Overcoming Limitations In Current Pre-Transfusion Compatibility Testing Methods Using Phage Display Technology

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OUTLINE OF PRESENTATION

- Drawbacks of current pre-transfusion testing methods
- Overview of phage display technology
- Use of phage display to create “conventional” agglutination-based antibody reagents
- Use of phage display to create novel “genetic-based” antibody reagents

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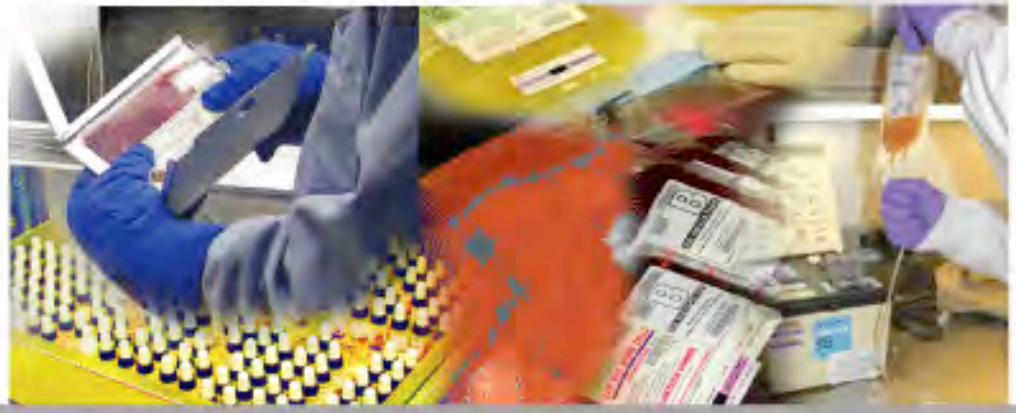
PhenoTech develops novel blood typing reagents as well as innovative therapeutic agents for the treatment of various hematologic and cardiovascular disorders. PhenoTech uses its proprietary phage display technologies to rapidly create and develop unique monoclonal antibodies with diagnostic and therapeutic applications.



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Our Products

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What's New

PhenoTech presents novel blood typing technology at AABB meeting

New Scientific Advisory Board announced

Terms and Conditions

Designed by Osiris Group, Inc.

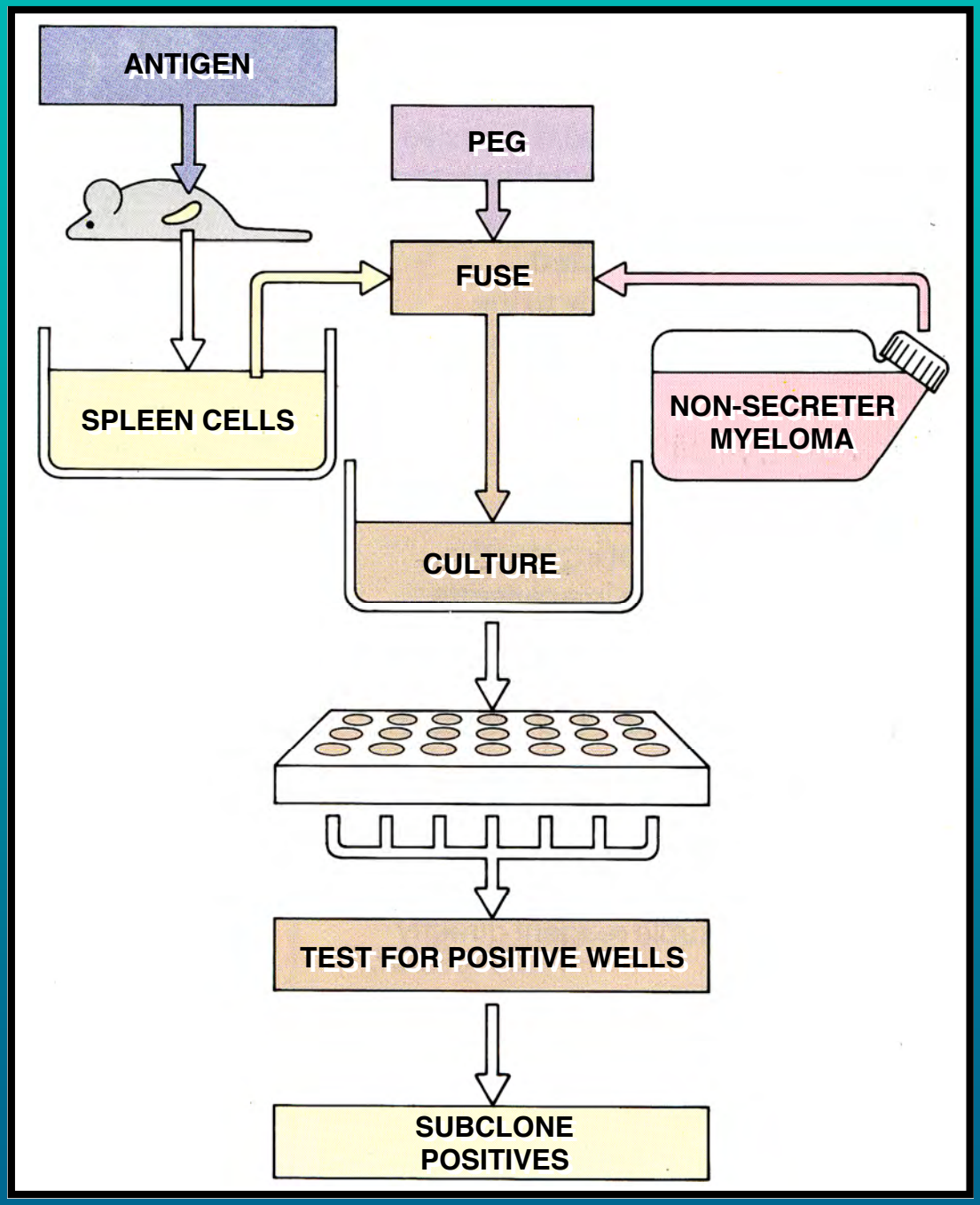
Current Pre-Transfusion Testing Methods

- Need reagents and methods in which to use them
- Reagents currently comprise anti-RBC antibodies, anti-human globulin, and reagent red cells
- Methods currently utilize agglutination (or some variant) as read-out

Current Pre-Transfusion Testing Methods

- Drawbacks of current methods
  - expense and, in some cases, scarcity of antibody reagents
  - method impractical for performing extending phenotyping on routine basis
    - reason for “reactive” vs. “proactive” practice of TM
    - medically can lead to:
      - delayed hemolytic transfusion reactions
      - delays in providing blood (positive screen leads to need to perform ab ID, then need to ID ag-negative units on the spot, then perform full-crossmatches vs. computer crossmatch, etc.)
    - financial impact of alloimmunization: 55% of pre-transfusion testing costs spent on working up ~15% of patients

Hybridoma Technology



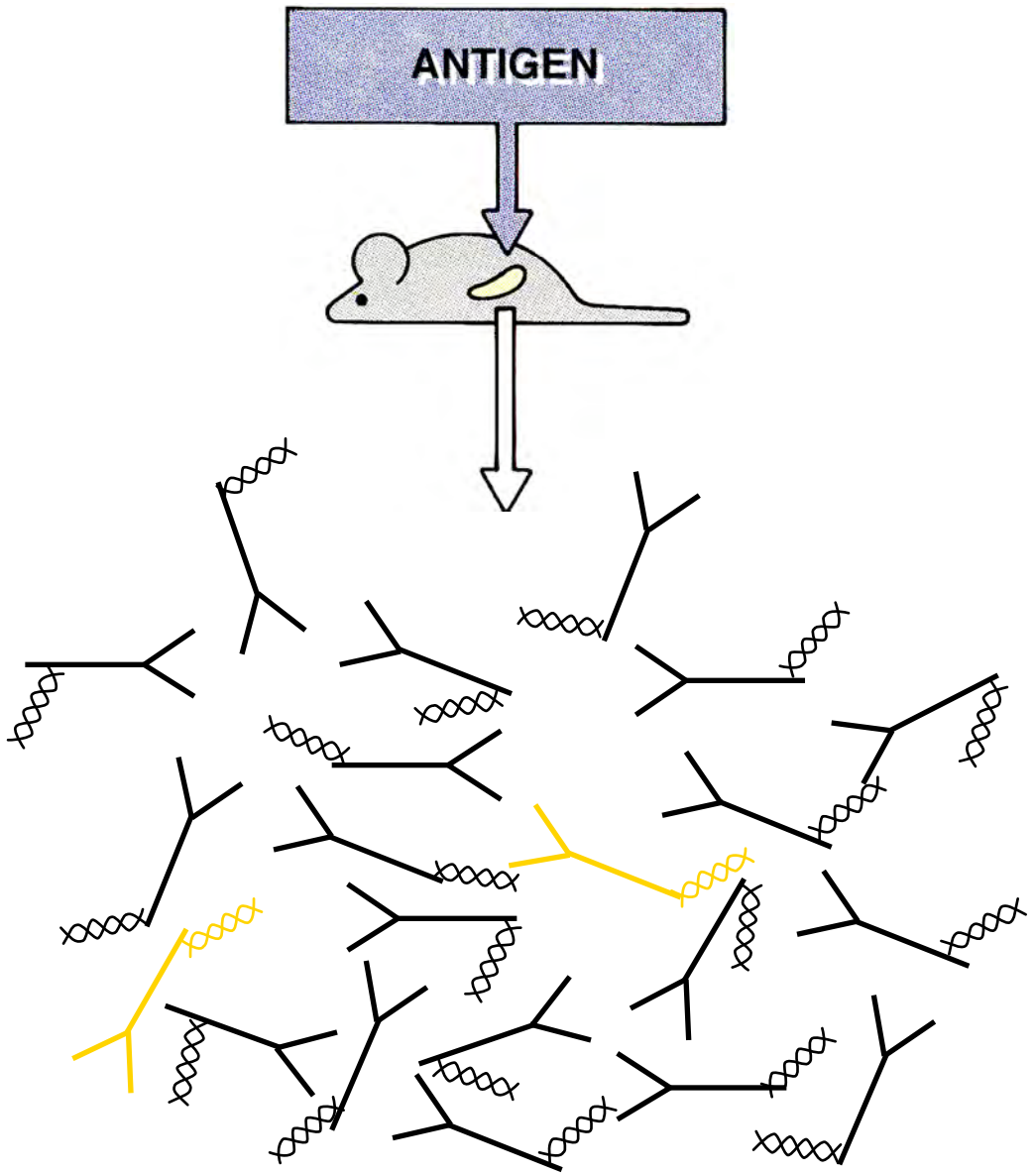
LIMITATIONS

- labor intensive
- expensive
- inefficient
- get what you get
- antibodies not human

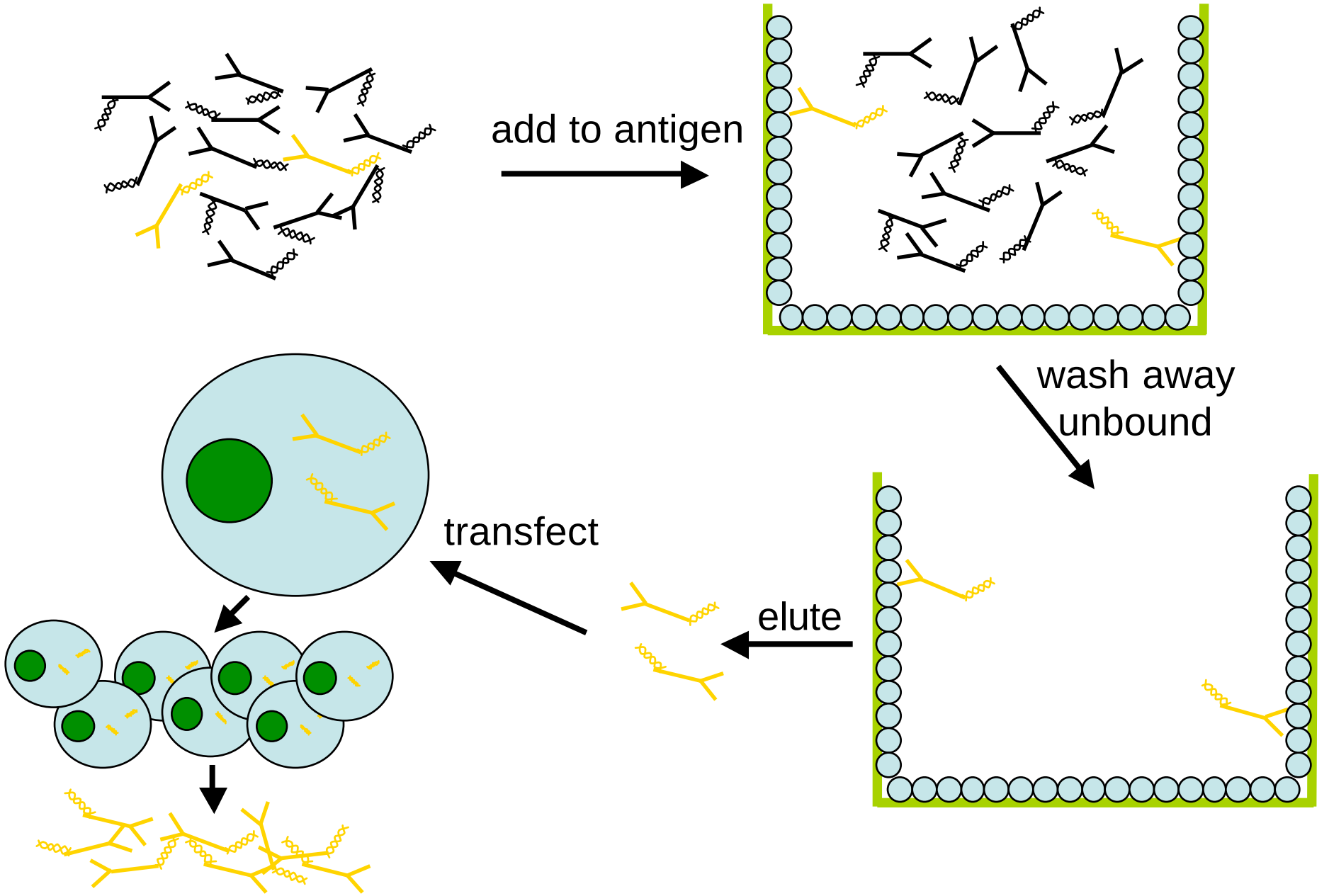
Problems With Conventional Methods for Production of Human Monoclonal Antibodies

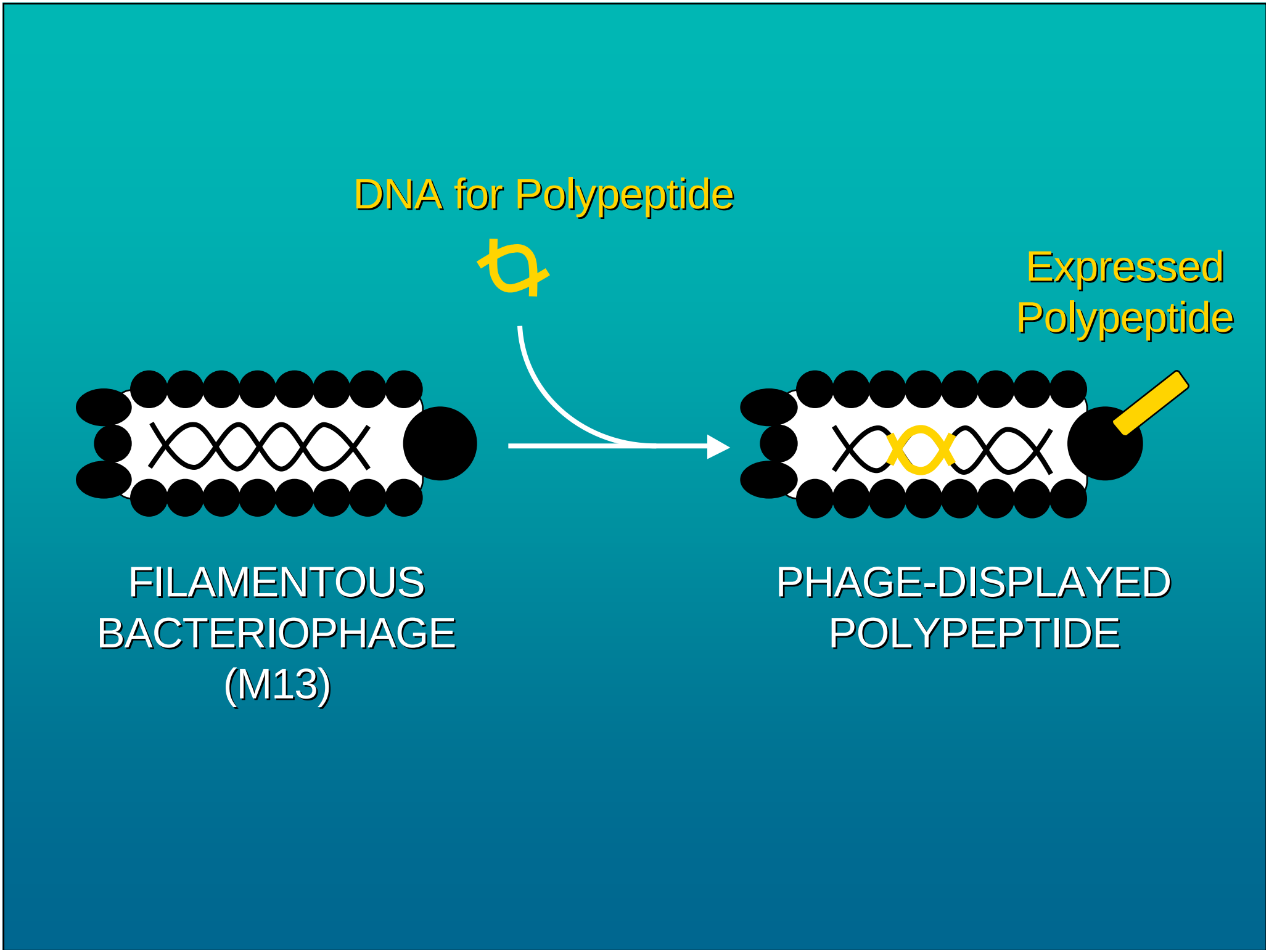
- low efficiency when using EBV-transformation approach
- low fusion frequency if attempt to make heterohybridomas
- decline in antibody production and growth
- instability of human/mouse heterohybridomas with progressive loss of human chromosomes

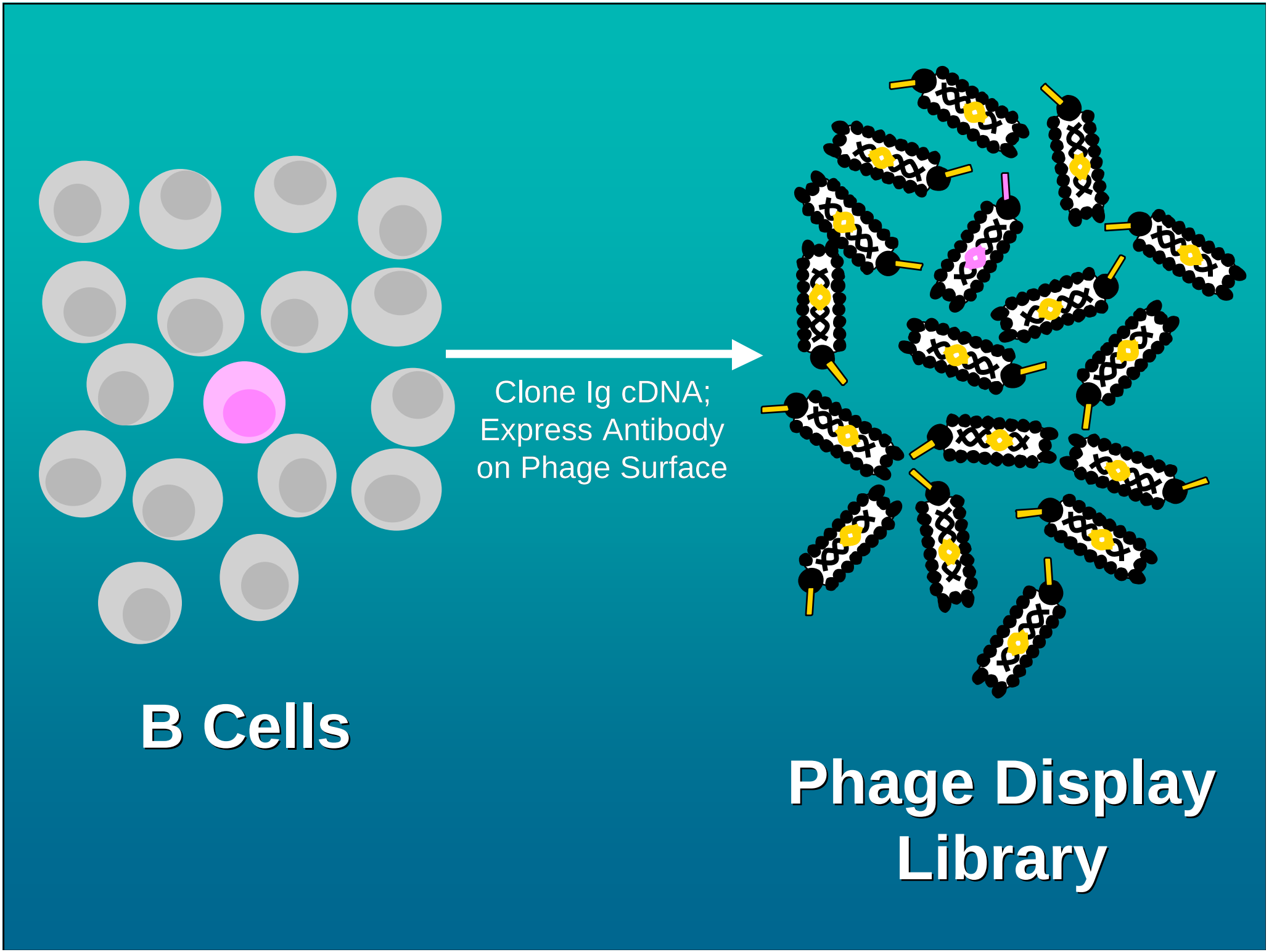
Science Fiction

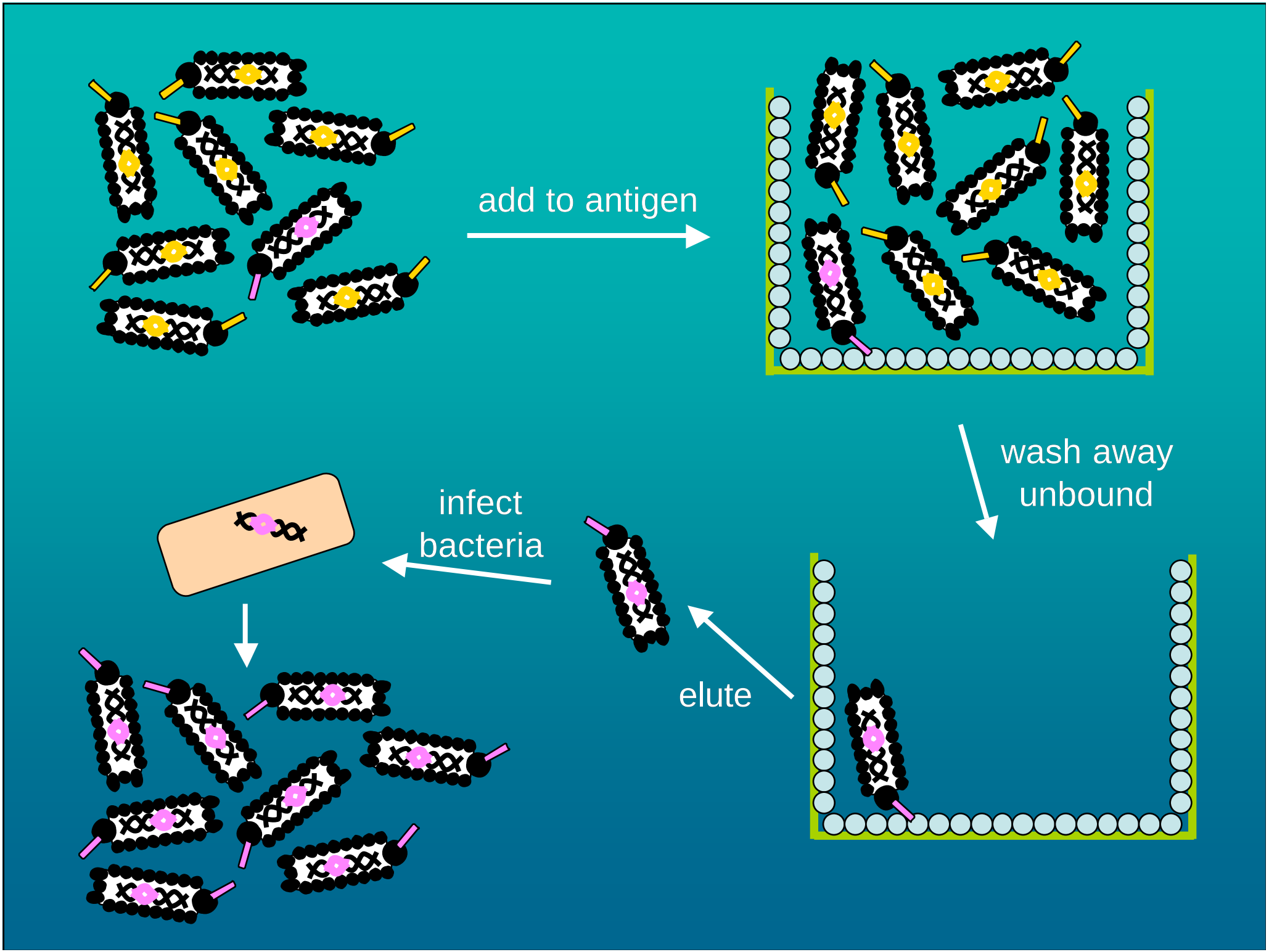


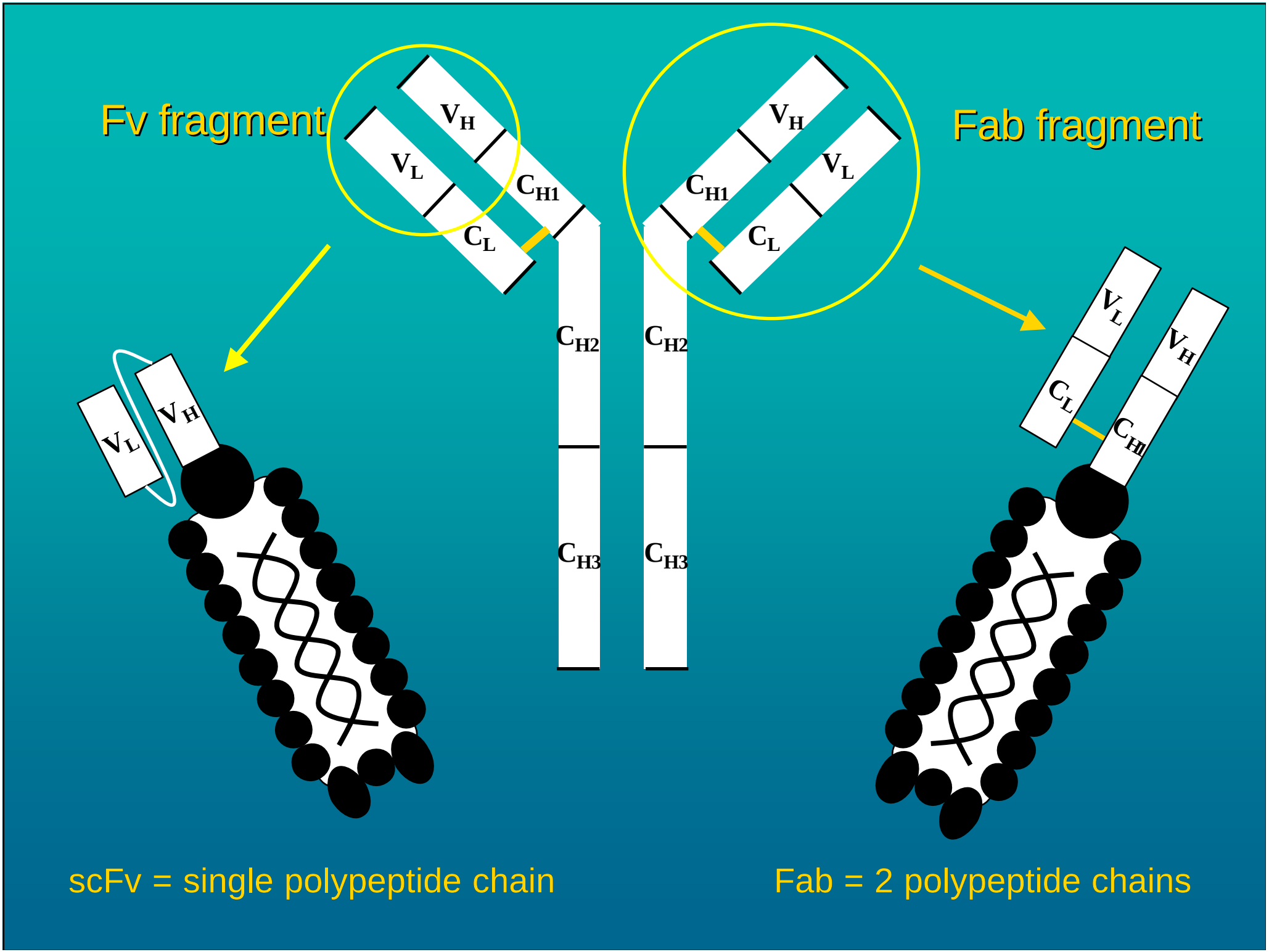
Science Fiction (cont.)

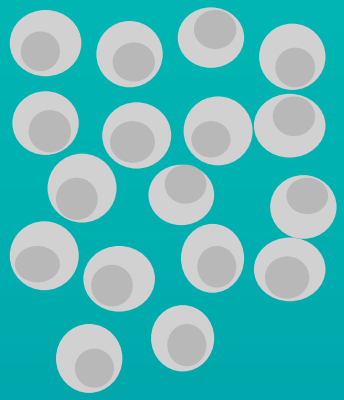



DNA for Polypeptide
Expressed Polypeptide
FILAMENTOUS BACTERIOPHAGE (M13)
PHAGE-DISPLAYED POLYPEPTIDE

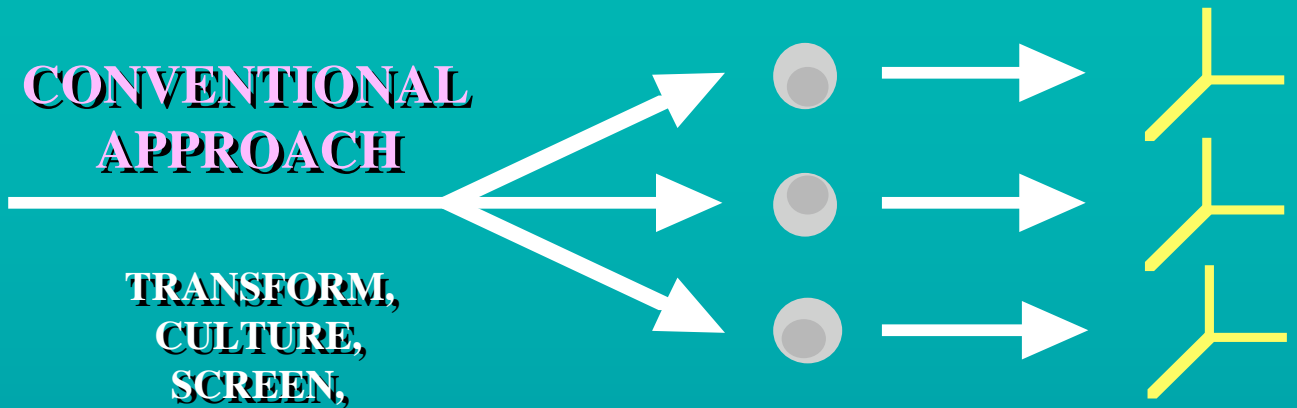

Clone Ig cDNA;
Express Antibody
on Phage Surface
B Cells
Phage Display
Library


add to antigen
wash away unbound
elute
infect bacteria


Fv fragment
Fab fragment
V_H
V_L
C_{H1}
C_L
C_{H2}
C_{H3}
scFv = single polypeptide chain
Fab = 2 polypeptide chains

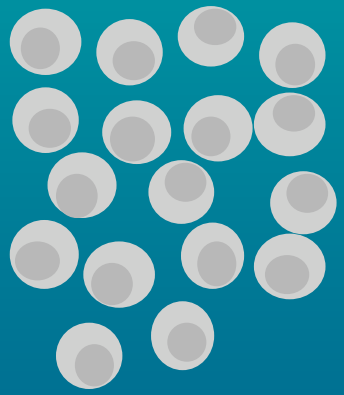


CONVENTIONAL APPROACH



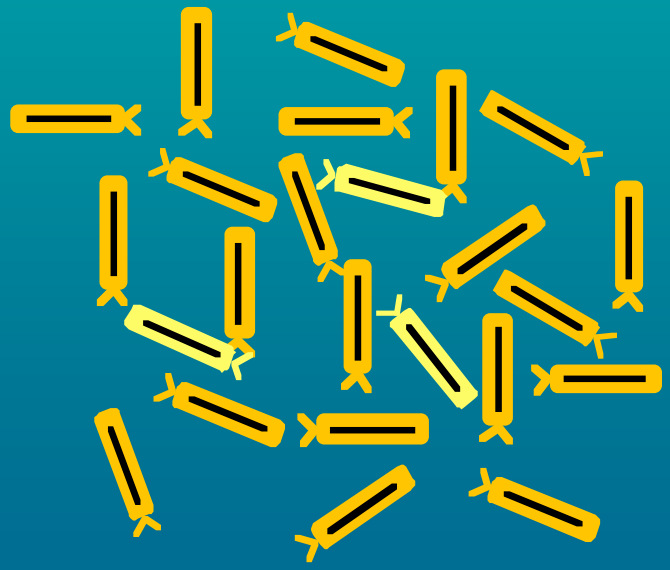
TRANSFORM, CULTURE, SCREEN, SUBCLONE, CULTURE, SCREEN, ETC.

B Cells



PHAGE-DISPLAY APPROACH

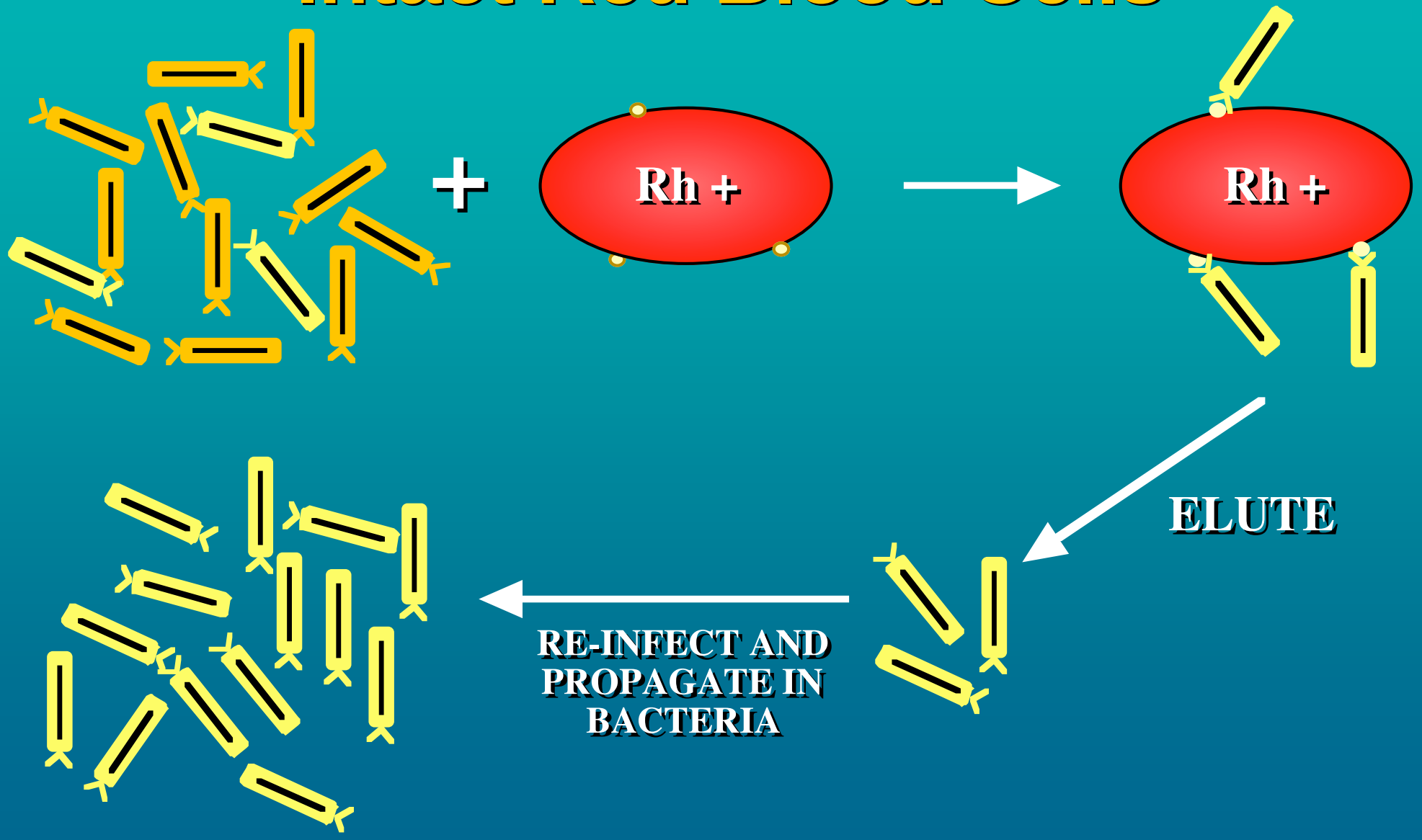
CLONE Ig cDNA, EXPRESS FAB ON PHAGE COAT



Advantages of Molecular Methods

- does not rely on immortalization of lymphocytes
- easily adapted to produce mAbs from any species (rabbit, chicken, monkey, camel, mouse, human)
- RNA-based, so access to all B-cell compartments
- isotype controllable/affinity-controllable
- streamlined screening and rapid production
- indefinitely stable and capable of self-replication

Panning Phage Libraries with Intact Red Blood Cells

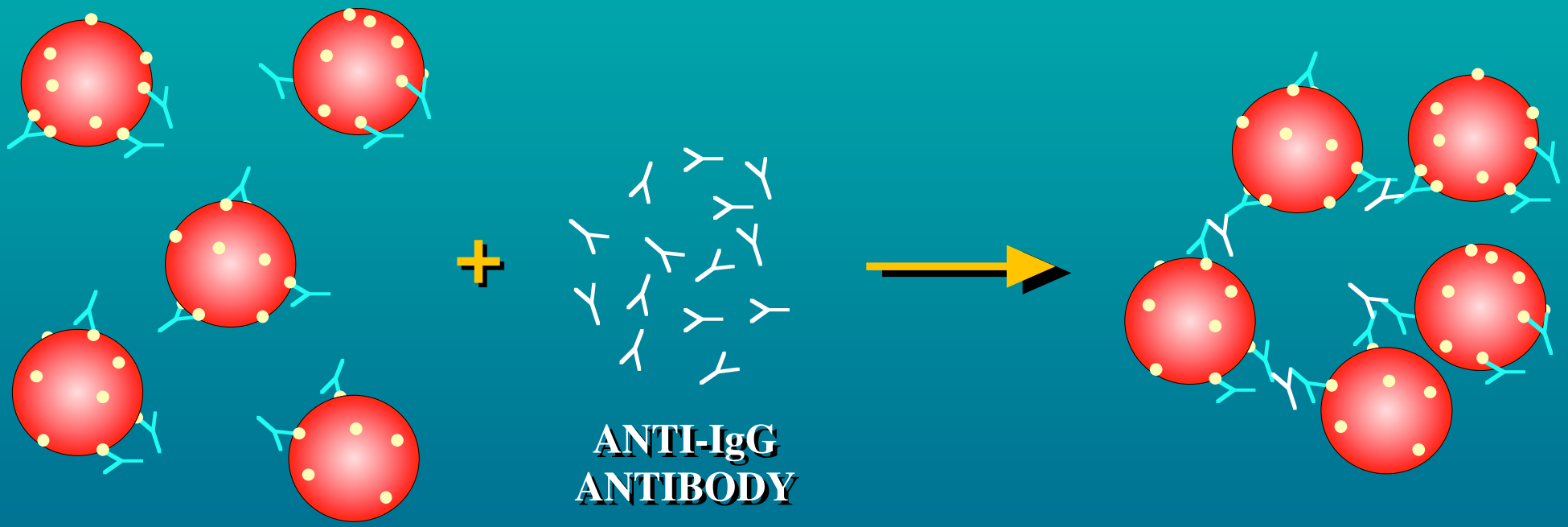


Yield of Anti-Rh Antibodies from a Single Experiment

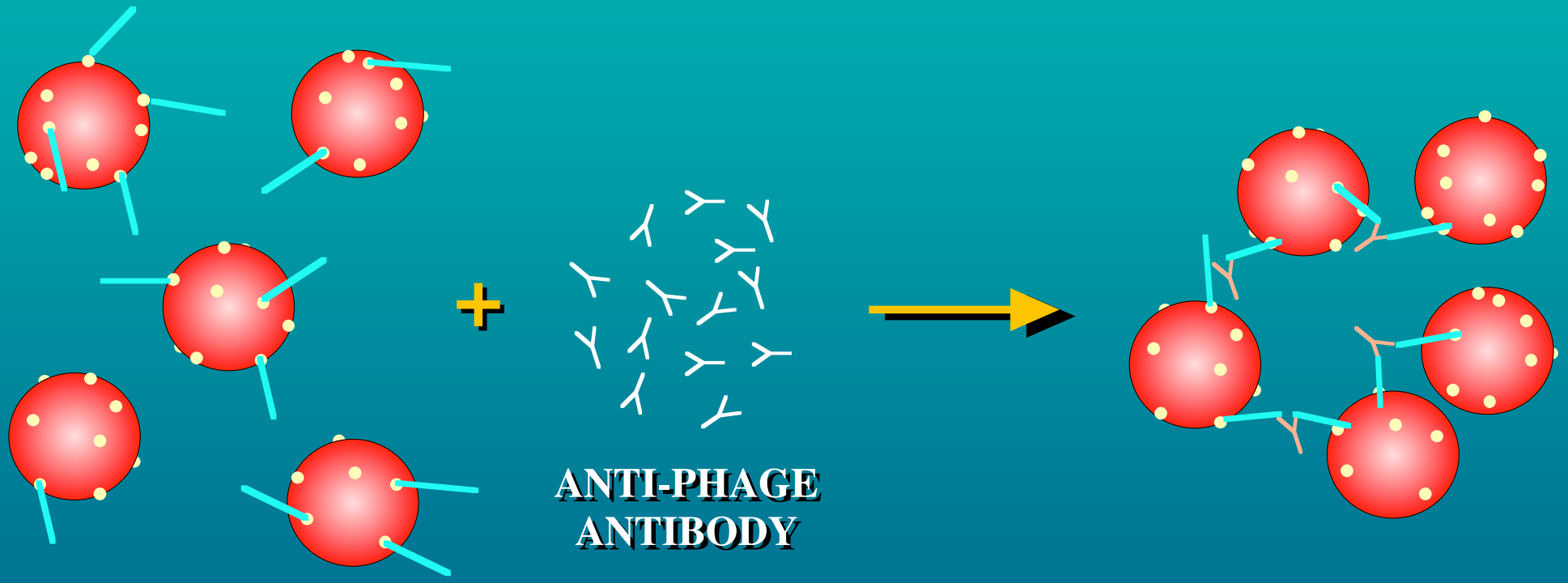
Sampled 83 clones (out of >10^6 anti-Rh(D) clones):

# of unique heavy chains	28
# of unique kappa light chains	18
# of unique lambda light chains	23
# of unique anti-D antibodies	53

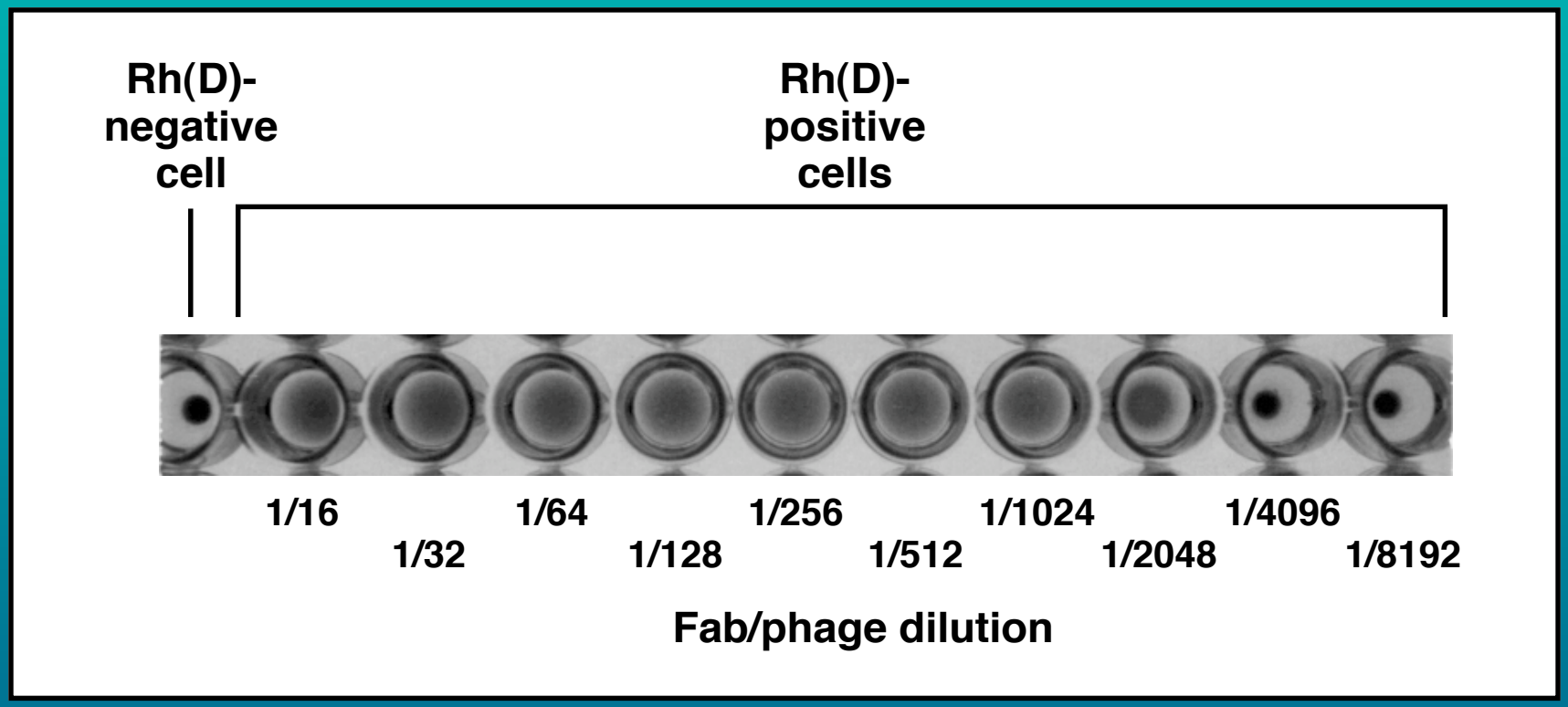
Blood Typing with Conventional Antibodies and Anti-IgG Antibodies

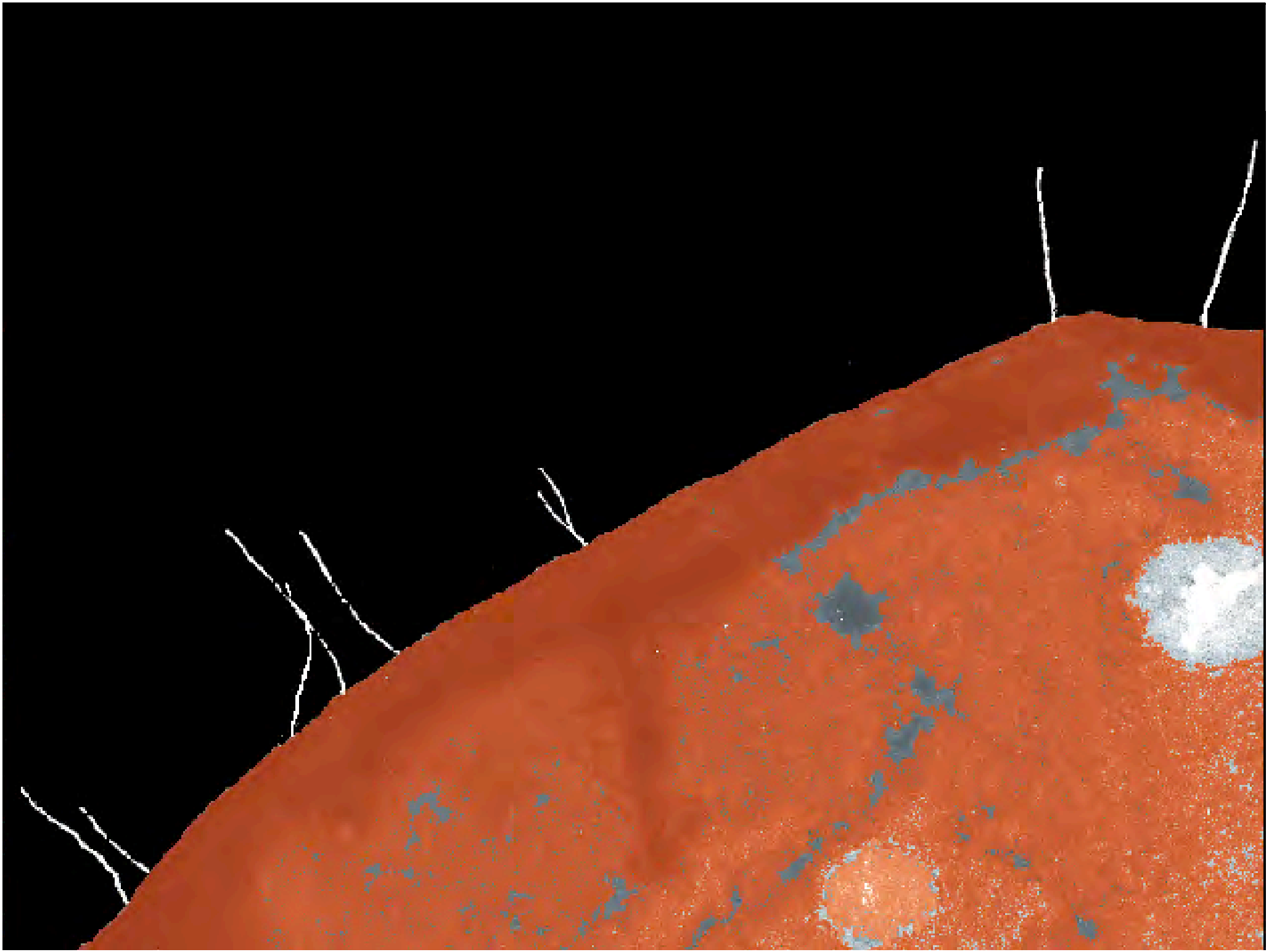


Blood Typing with Phage and Anti-Phage Antibodies



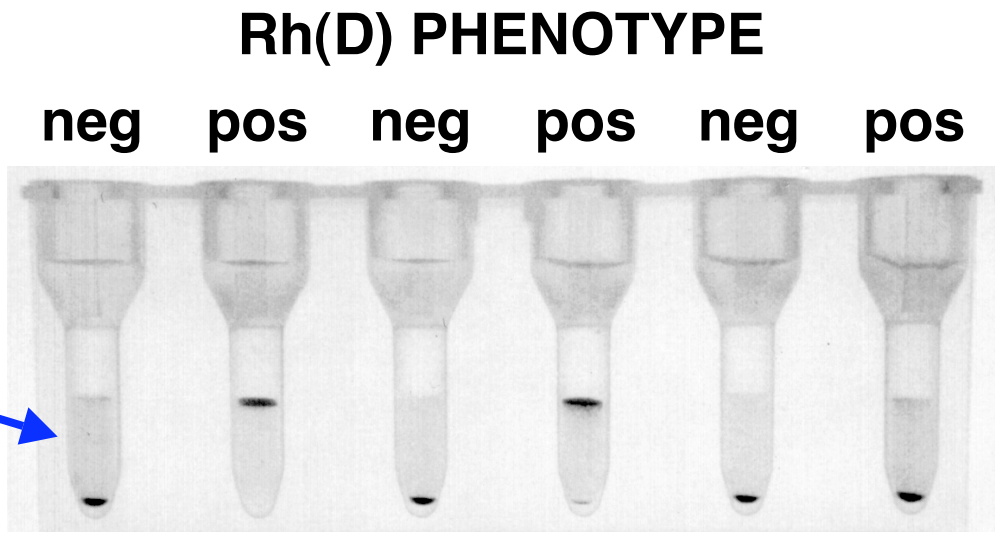
Phage-Displayed Antibodies as Blood Typing Reagents





Gel Card Assay with Phage-Displayed Antibodies

gel suspended in anti-M13 phage IgG (vs. anti-human globulin)



# Fab/phage added (x 10^7 cfu's):	100	100	20	20	4	4
# RBCs added (x $10^{7)}$:	1.6	1.6	1.6	1.6	1.6	1.6
RATIO Fab/phage per RBC:		63		13		2.5